#3 A P0 8/29/02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of:

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Serial No.: 09/741,426

Group Art Unit: 1634

Filed: December 21, 2000

Examiners: Goldberg

For: Method and Kit for Quantitating Genomic DNA Damage and Repair Capacity

August 22, 2002

AMENDMENT

(Request for Automatic Three-Month Extension)

Commissioner of Patent
and Trademarks
Washington, D.C. 20231

Sir:

Applicant requests an automatic three-month extension of time. An extension fee accompanies this request.

In reply to the Office Action mailed February 22, 2002, please amend the above-identified application, as follows:

In the Claims

Cancel claims 12-13 and 16-17, without prejudice of disclaimer.

Replace claims 1-11 and 14-15 with the following substitute claims 1-11, 14-15, and new claims 18-19. A redline version of these claims is attached in a separate paper.

A1

1. (Amended) A method of assaying sample DNA comprising:

(i) separately mixing a surface treatment solution with the sample DNA and with control DNA to form respective mixtures, said control DNA having a known extent of abasic sites,

(ii) depositing the mixtures of sample and control DNA at respective regions of an analysis plate to bind the sample DNA and control DNA to the plate,

(iii) reacting abasic sites of the sample DNA and the control DNA with an aldehyde group-specific chemical reagent whereby to attach the reagent to abasic sites, and

(iv) measuring the extent of abasic sites tagged with biotin after the reacting step.

2. (Amended) The method as recited in claim 1, comprising, prior to the depositing step, reacting the reagent with abasic sites of DNA of cells in culture

3. The method as recited in claim 1, wherein the control DNA is a depurination of calf thymus.

4. (Amended) The method as recited in claim 1, wherein the steps performed relative to the sample DNA and the control DNA are performed simultaneously so as to remove environmental or process variables.

5. (Amended) The method as recited in claim 1, wherein the surface treatment solution used in the mixing step comprises one of Reacti-bind and Protomine Sulphate.

6. (Amended) The method as recited in claim 1, wherein the aldehyde group-specific chemical reagent in the reacting step comprises an aldehyde reactive probe (N'-aminooxymethylcarbonylhydrazino-D-biotin).

Sub C7 7. (Amended) A method of quantitatively assaying damage of sample DNA having abasic sites, said method comprising the steps of depositing on an analysis plate respective surface treatment solutions containing sample DNA and multiple control DNA specimens wherein each control DNA specimen has a known extent of abasic sites, binding residues of

A' contd

the sample DNA and the control DNA specimens to the analysis plate by removing unbound DNA and excess surface treatment solutions, tagging aldehyde groups associated with abasic sites of the sample and control DNA bound to the analysis plate, providing an indication of tagged abasic sites of the sample DNA and control DNA specimens bound to the analysis plate, and comparing the sample DNA with multiple control DNA specimens to determine the extent of abasic sites in the sample DNA.

8. (Amended) A method of assaying repair capacity of a sample enzyme wherein the repair capacity is indicated by results of enzyme activity acting on DNA lesions that produces abasic sites, said method comprising:

treating respective DNA specimens with said sample enzyme and a control enzyme to produce respective substrates to which a probe may attach, said DNA specimens having lesions and said control enzyme having a known DNA repair capacity,

depositing on an analysis plate respective surface treatment solutions containing the DNA specimens,

binding to the analysis plate residues of the DNA specimens by removing unbound DNA and any excess of the surface treatment solutions,

reacting the DNA specimens with the probe thereby to tag aldehyde groups associated with abasic sites formed on the DNA specimens,

providing an indication of tagged abasic sites of the respective DNA specimens bound to the plate, and

comparing the indication of the DNA specimen treated with the sample enzyme with the indication of the DNA specimen treated with the control enzyme to determine relative enzyme activity levels of the sample and control enzymes, whereby to provide an indication of repair capacity of the sample enzyme.

9. (Amended) The method as recited in claim 8 wherein the repair enzyme is selected from the group including Endonuclease III, 8-oxoguanine glycosylase [yOGG1], human 8-oxoguanine glycosylase [hOGG1].

10. (Amended) A method of assaying sample DNA relative to control DNA that has abasic sites, said method comprising:

A1 concld

(i) binding sample and control DNA to a microtiter plate by respectively mixing a Reacti-bind solution with sample and control DNA, and then depositing solutions containing the sample and control DNA on the microtiter plate,

(ii) removing from the microtiter plate any excess Reacti-bind and unbound DNA using a detergent so as not to remove the bound DNA,

(iii) while bound to the plate, reacting the bound DNA with an excess amount of aldehyde reactive probe (ARP) reagent,

(iv) removing the excess and unreacted ARP from the microtiter plate,

(v) tagging the attached ARP using a biotinylated chemical agent, and

(vi) performing analysis of the sample and control DNA attached to the plate to quantitatively assay by comparison of relative the extent of abasic sites in the sample DNA relative to the control DNA.

11. (Amended) The method as recited in claim 10, wherein the binding step includes binding to the analysis plate a relatively high percentage of DNA contained in a low concentration solution having a concentration range of 1.0 to 10.0 micrograms of DNA per milliliter.

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14. (Amended) A method of determining DNA repair capacity of a substrate specific repair enzyme comprising the steps of:

separately subjecting DNA specimens to a control enzyme and to the substrate specific repair enzyme,

tagging [the product of the] abasic sites resulting from enzyme reaction with a probe,

binding the DNA specimens to an analysis plate by forming respective mixtures of the DNA specimens and a surface treatment solution, depositing the respective mixtures on the analysis plate, and subsequently removing any excess surface treatment solution and unbound DNA specimens,

determining the extent of abasic sites in the respective DNA specimens based on the extent of tagging whereby to assay the effectiveness of the DNA repair enzyme relative to the control enzyme.

A2 cont'd

15. The method as recited in claim 14, further comprising the steps of subjecting the sample and control DNA to a DNA glycosylase selected from the group of endonuclease III, N-glycosylase, 8-oxoguanine, alkA protein, and other broad and narrow spectrum DNA glycosylase.

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18. (New) The method of claim 15, wherein said surface treatment solution comprises Reacti-Bind.

19. (New) The method of claim 14, wherein the subjecting and tagging steps occur after the binding step.

20. (New) The method of claim 1, wherein the reacting step occurs before the separately mixing step.

REMARKS

Applicant appreciates the examiner's thorough examination of the application, as reflected by the rather extensive action.

I. Election

Applicant affirms the election of Group I claims 1-11 and 14-15, which was made by telephone on November 5, 2001.

II. Information Disclosure Statement

Since it appears that the examiner has applied the most relevant art, some of which being identified in the present application, applicant believes filing a formal Information Disclosure Statement would only be superfluous.

III. Specification

Applicant is in the process of preparing a substitute specification to reflect editorial changes and to correct minor typographical errors. A new substitute specification will be submitted upon indication of allowable subject matter. However, in reply to the examiner's

current objection, a substitute page 9 that deletes the embedded hyperlink is submitted herewith.

IV. Priority

The examiner has challenged applicant's claims of priority based on Provisional Application 60/171,309 filed December 21, 1999. The examiner "invites the applicant to point to support" in the Provisional Application, but applicant finds it difficult to respond because the examiner has failed to identify (i) which claims are purportedly unsupported by the Provisional Application or (ii) any specific element of any claim that purportedly is not supported by the Provisional Application. See 37 CFR §104(a)(2) (the examiner is required to provide "such information ... as may be useful in aiding the applicant" to respond to the action). Accordingly, applicant is unable to determine whether the objection to the priority claims lies with all claims, a single claim, or a single element contained in the claims.

As to where the objection lies, a slight hint is provided in paragraph 11 of the examiner's comments where it is stated that claims 1-3 and 5-7 may be unsupported. Without the benefit of knowing specifics of the purportedly unsupported matter, applicant blindly points to the attached page 4 of the Provisional Application that sets forth *Immobilization of DNA to the Microtiter Plate* since this aspect, among other things, is relied upon as a distinction over the cited and applied art. Here, it is seen that the Provisional Application discloses a DNA-Reacti-Bind solution being formed and then placed (and or incubated) in respective wells of an analysis (microtiter) plate in order to efficiently bind the DNA to the plate. Makrigiorgos (U.S. Pat. 6,174,680) simply discloses use of a Reacti-Bind *coated plate*, but not a *mixture* of DNA and in a Reacti-Bind *solution*, as provided in the amended claims. The Provisional Application advantageously provides that the solution

mixture enables binding of 50-400 ng of DNA in wells of the microtiter plate and the present application, at page 13, lines 20-12, provides that 100-360 ng of DNA may be bound. This contrasts with 70-100 ng of DNA bound using Kubo's process (Kubo, page 3704, col. 2). Makrigiorgos does not specify the any DNA is bound to a plate.

Accordingly, at least this distinctive aspect is supported by the Provisional Application. Applicant reserves the right to supplement this response should the examiner specifically identify what is not allegedly supported.

V. Rejection Under 35 USC §112, First Paragraph

The examiner's rejection of claim 11 appears to have been predicated on a mistaken recital in the claim, and is therefore well taken. By the above amendment, the term "nanogram" has been corrected to "microgram," as supported at page 12, line 30, of the specification.

VI. Rejection Under 35 USC §112, Second Paragraph

Regarding paragraph A of the examiner's comments, applicant amended claim 1 to recite that the reagent is reacted with both control and the sample DNA. In addition, application removed the Markush language.

Regarding paragraph B, claim 2 has been amended to correct a typographical error.

Regarding paragraph C, the labeling step of claim 6 has been corrected to a "reacting" step.

Regarding paragraph D, claim 7 has been amended to clarify that only the control DNA has a known extent of abasic sites. Claim 7 has been further amended to recite that the Elisa-like method yields an "optical indication," rather than one of absorbance, density, and color.

Regarding paragraph E, claim 8 has been amended to clarify how repair capacity is indicated by number of abasic sites in response to enzyme activity.

Regarding paragraph F, the preamble has been expanded and recitals of claim 10 have been clarified.

Regarding paragraph G, claim 11 has been amended to recite a modification of the binding step. Also, the language of claim 11 has been clarified to recited an aspect of the invention enabling efficient binding of DNA to a plate.

Regarding paragraph H, the preamble and body of claim 14 has been amended to better recite determining repair capacity. The determining step has also been clarified. As known in the art, a substrate specific repair enzyme acting on DNA also produces abasic sites. Such sites can also be detected using the methods of the invention. If the repair enzyme is not effective, this is reflected in a reduced number of abasic sites after repair enzyme activity. This is further described at Sec. B, pp. 166-167, of the Kow and Dare referenced cited by the examiner.

VII. Prior Art Rejections

The examiner has asserted multiple prior art rejections under §§102 and 103. The rejection, in substantial part, stemmed from a failure on applicant's part to clearly set forth a crucial distinctive feature of the invention. Applicant believes the amend claims distinctively define the invention over the cited art.

In particular, claim 1 recites separately mixing sample and control DNA with a surface treatment solution, depositing the resulting mixture on the analysis plate to bind the DNA, and then reaction the DNA with a probe. The surface treatment solution may comprise Reacti-Bind, Protomine Sulphate, or other surface treatment solution.

(Specification, p. 20). Applying the DNA to the analysis plate in a surface treatment solution enables binding a high percentage of DNA suspended in the solution, thus advantageously allowing one to obtain a DNA sample from a very low concentration DNA source, e.g., the bucca epithelium. (Specification, p. 6, line 7). Over 90% binding efficiency is achieved. (Provisional Appln., page 5)(Attached). None of the prior art discloses use of such a DNA mixture in a surface treatment solution to achieve binding.

Makrigiougos (U.S. Pat. 6,174,680), for example, only discloses use of Reacti-Bind *coated* analysis plate to which DNA may be bound. Kubo (Biochemistry 1992) discloses irradiating the analysis plate to improve binding efficiency, but does not disclose mixing DNA with a surface treatment solution and depositing that solution on the plate to effect binding, as now recited in claim 1. As clearly stated in Kubo, on 70-100 ng of DNA was bound, which contrasts with 100-360 ng of DNA achieved by the present invention. Such higher binding efficiency enable detecting of lower concentrations of abasic sites.

The only cited reference disclosing a mixture of DNA and a surface treatment solution is Kow, which is non-statutory prior art due to its 2000 publication date. Applicant claims priority from his provisional application filed 1999, where at p. 4 (attached), the DNA-Reacti-Bind solution mixture is described.

Claims 7, 8, and 10 are further distinguished by reciting, after depositing the solution containing the sample and control DNA, removing unbound DNA and/or excess surface treatment solution. None of the cited art meets this limitation because none uses a mixture of DNA and surface treatment solution to deposit DNA on an analysis plate.

Claim 14 is distinguished because none of the art discloses a method of determining repair capacity of an enzyme. Use of the efficient binding process to provide an indication of

repair capacity was disclosed in applicant's Provisional Application (See Abstract, Attached). In addition, the Kow reference cannot be applied under §102(a) because it is applicants own work. An affidavit will be submitted to confirm this fact.

VIII Conclusion

Kow, in addition to being applicant's own work, is non-statutory due to its 2000 publication date.

None of the valid references disclose mixing DNA with a surface treatment solution, and binding DNA by depositing the resulting mixture on an analysis plate, and therefore, do not meet the limitation of the claims, as amended.

Reconsideration is respectfully requested.

Respectfully submitted,

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Abstract

Several methods for quantifying AP sites have been reported, allowing discrimination of low AP sites in DNA. However, some of these methods require radioactivity. Others not requiring radioactivity are either extremely time consuming, technique-sensitive, or cumbersome. We developed a rapid, simple, sensitive and cost-effective method to detect and quantify abasic sites in DNA by reacting an aldehyde group-specific biotinylated chemical reagent called Aldehyde Reactive Probe (ARP) with AP sites in DNA directly on the microtiter plate. This unique method obviates the need for ethanol precipitation or ultrafiltration to remove excess chemical probe from DNA before adding to plate, in variance to previous ARP methods. The biotin-tagged AP site was detected and the number determined colorimetrically using avidin-biotin-horseradish-peroxidase conjugate method. We used Reacti-bind DNA coating solution (Pierce Chemical, Rockford, Illinois, and U.S.A.) to immobilize DNA to the microtiter plate. An enhanced and consistent binding of DNA to microtiter plate was achieved. The assay was able to determine accurately, specific AP sites in calf thymus DNA generated by acid/heat depurination method, and spontaneously generated AP sites by incubation at physiological conditions (pH 7 and 37 C). The new Direct ARP assay was sensitive enough to measure 0.5 AP/10^5 bp (1 AP site/5 x 10^6 bases) in 190 ng of double stranded DNA bound to plate. This current development is easy, rapid and inexpensive method to determine the number of AP sites in human genomic DNA, with possibility of automation for large number of samples. Moreover, when this method is N-glycosylase enzyme-coupled, it could be adapted to estimate DNA repair capacity in cells and tissues using the principle of enzymatic processing.

Introduction

The formation of abasic sites (AP sites) resulting from the removal of purine or pyrimidine bases is among the most common oxidative lesions in DNA. Endogenous and exogenous processes can give rise to abasic sites. In humans, it was estimated that about 10 000 AP sites are produced per cell every day by hydrolysis of the N-glycosylic bond under physiological conditions (1). Ionizing radiation (2), potent carcinogens, chemical agents such as bleomycin (3) and alkylating agents (4) also promote the formation of AP sites. In addition, AP sites are intermediates in the base excision repair pathway, where damaged base is being removed by DNA N-glycosylases as the first step in base excision repair process (5). Deficiency in the repair pathway and increasing oxidative stress could contribute to increased background levels of AP sites. Since AP site lesion was shown to be strong block to DNA synthesis in vitro (6), AP sites accumulation could cause cell death and/or mutation induction (7,8). Accumulating evidence from recent studies implicate increased background level of oxidative DNA base damages in the pathogenesis of some human diseases. These include Alzheimer's disease (9), amyotrophic lateral sclerosis (10), Parkinson's disease (11), cataract formation, aging process (12) and some types of cancers (13,14). Cells and human tissues are also being screened for specific DNA damage in order to correlate the action of toxic agents with human diseases (15,16,17,18).

The biological significance of AP site enumerated, stimulate interests in developing specific and sensitive methods to detect and quantify abasic sites in DNA. Although several methods for measuring AP sites have been reported, many, when sensitive enough to measure low-level AP sites require radioactivity (19). Others, while not requiring radioactivity are either not sensitive (20), or do require costly equipment, skill-sensitive and time-consuming (21,22). A previous method attempting a solution to these challenges used Aldehyde Reactive Probe (ARP) to tag biotin to the aldehyde group of AP sites in DNA (23). The ELISA-like method reported promised to be of advantage in its specificity, but was limited in its sensitivity. Attributable to this limitation was two major problems. First, there is limitation of DNA binding to the UV-irradiated microtiter plate. Second, there is non-specific binding of ARP to the UV-irradiated microtiter plate. These cause inconsistent measurement and high background noise respectively, reducing the reliability of measurements. Another draw back was the laborious, but important step of ethanol precipitation to remove excess ARP that limits the use of the assay when available DNA sample is small. The current development allows rapid and sensitive measurement of AP sites in DNA directly on the microtiter plate, obviating the need for ethanol precipitation or ultrafiltration/centrifugation. We describe the development of a new non-isotopic microtiter plate-based chromogenic method to detect ARP-tagged aldehyde-containing AP sites in DNA. Subsequently, we demonstrate the application of the method to detect and measure AP sites in calf thymus DNA generated by heat/acid-buffer depurination (21), and spontaneously by incubation under physiological conditions (1,22).

Materials and Methods

Heat/Acid-Buffer Depurination of Calf thymus DNA.

We purchased pure grade double stranded calf thymus DNA from Sigma Chemical Co. Specific number of AP sites were selectively produced in the DNA by heat/acid-buffer treatment as

previously reported (21). On another hand, prior to the heat/acid-buffer treatment, the DNA was treated with 5 mM methoxyamine for 1 h at room temperature in order to remove traces of existing aldehyde. The methoxyamine was then removed by ethanol precipitation and the sample re-suspended in sodium phosphate buffer, pH 7. The methoxyamine-treated and methoxyamine non-treated DNA (100 ug/mL) were then dialyzed separately in 10 mM NaH_2PO_4, 100 mM NaCl and 10 mM sodium citrate at pH 5.0 (AP-buffer). The dialyzed DNA was heated at 70 C for 50 minutes and the reaction stopped by chilling rapidly on ice to create 5 AP sites/10^4 bp (20). Each sample was dialyzed back to pH 7.5 in PBS buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4.7H_2O$, and 1.4 mM KH_2PO_4). This was appropriately diluted with control DNA to produce 1, 2, 3 and 4 AP sites/10^4 bp respectively. Appropriate DNA concentrations were also obtained by diluting with PBS buffer. To create relatively low-level AP site in methoxyamine treated DNA, 1 AP site DNA sample was appropriately diluted with control DNA to produce 0.1, 0.2, 0.4 and 0.8 AP site/10^4 bp respectively. Similarly, low-level AP site in non-treated DNA was created by appropriately diluting 2 AP-DNA sample with control DNA, to produce 0.2, 0.4, 0.8 and 1.6 AP site/10^4 bp respectively.

Immobilization of DNA to Microtiter Plate

In order to enhance binding of DNA to 96 well U-bottom high binding plate (Costar #3791-Costar corporation, Cambridge, MA), 200 uL of DNA at 1.25, 2.5, 5 and 10 ug/mL concentration were modified respectively with 300 uL Reacti-bind DNA coating solution (Pierce Chemical Corporation, Rockford, Illinois), resulting in 0.5, 1, 2, and 4 ug/mL DNA respectively after Reactibind modification. 100 uL of each mixture was added to each well respectively. This implies that, 50, 100, 200 and 400 ng of DNA respectively were introduced to the plate. The plate was incubated at room temperature overnight for 16 h. Unbound DNA was removed and plate was then washed three times with 0.1% Tween-PBS buffer (Phosphate buffered solution containing 0.1% Tween 20). Subsequent steps of the assay do not detach bound DNA nor alter the bound DNA.

The New Direct ARP Assay

In order to trap the open chain aldehyde generated in DNA at the position of AP sites, 100 uL of 1 mM of ARP was added to each well, and plate was incubated at room temperature for 1 h. After discarding contents, excess ARP in each well was removed by washing plate three times with 1% Tween-PBS buffer (Phosphate buffered solution containing 1% Tween 20), followed by washing with 0.1 % T-PBS once. The plate was swiped and dried without desiccating. 80 uL of 1:20 diluted ABC solution was then added to each well. The plate was covered with parafilm and incubated at 37 C for 1 h and then washed with 0.1 %Tween-PBS buffer three times. When Azinobis(3-ethylbenzo thiazoline-6-sulfonic acid) (ABTS) was used as substrate for horseradish peroxidase (HRP), 120 uL of the substrate prepared according to manufacturer's instruction was added into each well. After incubation at room temperature for 1 h, the absorbance was measured at 405 nm. When 3,3,5,5-Tetramethylbenzidine (TMB) (Moss Inc., Pasadena, Maryland, U.S.A.) was used as substrate for horseradish peroxidase, 160 uL of the substrate solution was added to each well and incubated at 37 C for 30 minutes. The absorbance was then measured at 650 nm. In all ARP assays, signals were expressed as the change in absorbance after subtracting background readings for control DNA. All experimental samples were in triplicates or more, with standard deviation less than 10 %.

Spontaneous Physiological Depurination of Calf Thymus DNA

AP sites were slowly generated in methoxyamine-treated calf thymus DNA by spontaneous depurination under physiological condition of pH 7.0 and incubation at 37 C for 4, 6, 8 and 10 days respectively. The number of abasic sites in the samples was then monitored using the present assay, the new direct ARP assay.

Results

Binding Efficiency of DNA to Plate

The amount of DNA bound to plate when DNA was immobilized using reacti-bind solution was determined by an ultrasensitive DNA quantitative fluorescent assay (PicoGreen) following the manufacturer's instructions. We determined the DNA concentration in each well after allowing binding overnight for 16 h. The amount bound was calculated from the difference before and after incubation, taking note of any change in volume after incubation. More than 90 % of DNA is bound when modified with Reactibind. The amount of DNA bound increased with time, while an optimum binding was ensured by 16 h. Figure 1 shows the amount of DNA bound to plate when DNA was modified by Reacti-bind DNA coating solution. There is a linear relationship between DNA bound and DNA concentration. When 5 and 10 ug/mL DNA were incubated for 16 h, 200 ng and 400 ng of DNA were in each wee respectively, and about 190 ng and 360 ng of DNA were bound to each well respectively. This indicates a binding efficiency greater than 90% was achieved.

AP Site determined by the New Direct ARP Assay

Figure 2 shows the ARP signal for selectively created AP sites in methoxyamine non-treated DNA at specific concentrations determined by the new direct ARP assay method using ABTS as horseradish peroxidase substrate. ARP signal increased with increasing AP sites in DNA. There is a linear relationship between ARP signal and DNA concentration. The sensitivity is higher for 10 ug/mL DNA than 5 ug/mL DNA (Table 1). At 10 ug/mL, when about 360 ng of DNA was bound in each well, the direct assay method was able to measure as low as 0.25 AP sites per 10^4 bp. At 5 ug/mL, when 200 ng DNA was started with and about 190 ng of DNA was bound in each well, the direct assay was able to measure as low as 0.4 AP site/10^4 bp.

Optimization of the New Direct ARP Assay for Low-level AP sites

Since obtaining 10 ug/mL of DNA from biological samples may prove difficult, requiring a large tissue mass source which may not be available, there is need to optimize the direct assay for smaller DNA concentrations. Moreover, higher sensitivity is required for determining low-level AP sites in DNA. In order to increase the sensitivity of the direct assay method, we used 3,3,5,5-Tetramethylbenzidine (TMB) (Moss Inc., Pasadena, Maryland, U.S.A.) in place of ABTS as horseradish peroxidase substrate. Figures 3A and 3B show the ARP signal for selectively created AP sites in methoxyamine non-treated DNA and methoxyamine-treated DNA respectively at specific concentrations, determined by the new direct ARP assay method. ARP signal increased with increasing AP sites in both treated and non-treated DNA. Also, there is a linear relationship between ARP signal and DNA concentration in both. The sensitivity is higher for 10 ug/mL DNA than 5 ug/mL DNA. The sensitivity increased with increasing DNA concentration and exceeds twice that of ABTS substrate (Table 1). Taken together, by varying DNA concentrations and horseradish peroxidase substrate, the new direct ARP assay can quantify very low AP sites in DNA.

REDLINE CLAIMS

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1. (Amended) A method of assaying sample DNA comprising:

(i) separately mixing a surface treatment solution with the sample DNA and with control DNA to form respective mixtures, said control DNA having a known extent of abasic sites,

(ii) depositing the mixtures of sample and control DNA at respective regions of an analysis plate to bind the [binding to an analysis plate both] sample DNA [under examination] and control DNA [having known abasic sites] to the plate,

[(ii)] (iii) reacting [the] abasic sites of the sample DNA and the control DNA with an aldehyde group-specific chemical reagent [selected from a group of reagents including an aldehyde reactive probe (ARP) (N'-aminooxymethylcarbonylhydrazino-D-biotin) reagent] whereby to attach the [ARP] reagent to abasic sites, and

[(iii)] (iv) [using an ELISA-like method to detect] measuring the extent of abasic sites tagged with biotin after the reacting step [wherein the ELISA-like method includes an avidin- biotin-complex conjugated with horseradish peroxidase or alkali phosphatase].

2. (Amended) The method as recited in claim 1, comprising, prior to the depositing step, reacting [ARP] the reagent with [AP] abasic sites of DNA of cells in culture [before the binding step.

wherein the sample and control DNA are tagged or labeled separately with a the residue of the ARP reagent and then bound to the analysis plate for comparison].

3. The method as recited in claim 1, wherein the control DNA is a depurination of calf thymus.

4. (Amended) The method as recited in claim 1, wherein the steps performed relative to the sample DNA and the control DNA are performed simultaneously so as to remove environmental or process variables [at the comparing step].

5. (Amended) The method as recited in claim 1, wherein the surface treatment solution used in the mixing step comprises one of Reacti-bind and Protomine Sulphate [is used during the binding step].

6. (Amended) The method as recited in claim 1, [further including a washing step after the binding and labeling steps] wherein the aldehyde group-specific chemical reagent in the reacting step comprises an aldehyde reactive probe (N'-aminooxymethylcarbonylhydrazino-D-biotin).

7. (Amended) A method of quantitatively assaying [DNA] damage of sample DNA having abasic sites, said method comprising the steps of [binding to] depositing on an analysis plate respective surface treatment solutions containing sample DNA and multiple control DNA specimens [each having of] wherein each control DNA specimen has a known [number] extent of abasic sites, binding residues of the sample DNA and the control DNA specimens to the analysis plate by removing unbound DNA and excess surface treatment solutions, tagging aldehyde groups associated with abasic sites of the sample and control DNA bound to the analysis plate, [performing an ELISA-like method to obtain one of absorbance, optical density, and color density] providing an indication of tagged abasic sites of the sample DNA and control DNA specimens bound to the analysis plate, and comparing the sample DNA with multiple control DNA specimens to determine the [number] extent of abasic sites in the sample DNA.

8. (Amended) A method of assaying repair capacity of a sample [DNA] enzyme wherein the repair capacity is indicated by results of enzyme activity acting on DNA

lesions that produces abasic sites, said method comprising:

treating [sample and control] respective DNA specimens with [an] said sample enzyme and a control enzyme [that] to produce[s a] respective substrates to which [ARP attaches] a probe may attach, said DNA specimens having lesions and said control enzyme having a known DNA repair capacity,

depositing on an analysis plate respective surface treatment solutions containing the DNA specimens,

binding to the analysis plate residues of the DNA specimens by removing unbound DNA and any excess of the surface treatment solutions,

reacting the [sample and specimen] DNA specimens with [ARP] the probe thereby to tag [tagging] aldehyde groups associated with abasic sites [of the sample and control] formed on the DNA specimens,

[performing an ELISA-like method to obtain one of absorbance, optical density, and color density] providing an indication of tagged abasic sites of the [sample DNA and control] respective DNA specimens bound to the plate, and

comparing [at least one of color, optical density, and absorbance of] the indication of the [sample] DNA specimen treated with the sample enzyme with [multiple] the indication of the [control] DNA specimen[s] treated with the control enzyme to determine relative enzyme activity levels of the sample and control [DNA] enzymes, whereby to provide an indication of repair capacity of the sample enzyme.

9. (Amended) The method as recited in claim 8 wherein the [treating step includes using an] repair enzyme is selected from the group including Endonuclease III, 8-oxoguanine glycosylase [yOGG1], human 8-oxoguanine glycosylase [hOGG1].

10. (Amended) A method of assaying sample DNA relative to control DNA that has abasic sites, said method comprising:

(i) binding sample and control DNA to [an analysis plate to] a microtiter plate [using] by respectively mixing a Reacti-bind solution with sample and control DNA, and then depositing solutions containing the sample and control DNA on the microtiter plate [wherein the control DNA has a known number of abasic sites],

(ii) removing from the microtiter plate [the] any excess Reacti-bind and unbound DNA using a [Tween 20 buffered] detergent so as not to remove the bound DNA,

(iii) while bound to the plate, reacting the bound DNA with an excess amount of aldehyde reactive probe (ARP) reagent,

(iv) removing the excess and unreacted ARP from the [analysis] microtiter plate,

(v) [labeling/tagging] tagging the attached ARP using a biotinylated chemical agent, and

(vi) performing [a colorimetric] analysis of the sample and control DNA attached to the plate to quantitatively [assess] assay by comparison of relative the extent of abasic sites in the sample DNA relative to the control DNA [attached to the plate].

11. (Amended) The method as recited in claim 10, [further including] wherein the binding step includes binding to the analysis plate a relatively high percentage of DNA contained in a low concentration solution [of relatively low concentration being in the] having a concentration range of 1.0 to 10.0 [nanograms] micrograms of DNA per milliliter.

14. (Amended) A method [for] of determining DNA repair capacity of a substrate specific repair enzyme comprising the steps of:

separately subjecting [sample and control] DNA specimens to a control enzyme and to [a] the substrate specific repair enzyme,

tagging [the product of the] abasic sites resulting from enzyme reaction with a probe,

binding the DNA specimens to an analysis plate by forming respective mixtures of the DNA specimens and a surface treatment solution, depositing the respective mixtures on the analysis plate, and subsequently removing any excess surface treatment solution and unbound DNA specimens ,

determining the [resulting number] extent of abasic sites [remaining on the analysis plate after the enzyme reaction] in the respective DNA specimens based on the extent of tagging whereby to assay the [ability of the cell to undergo] effectiveness of the DNA repair enzyme relative to the control enzyme.

15. The method as recited in claim 14, further comprising the steps of subjecting the sample and control DNA to a DNA glycosylase selected from the group of endonuclease III, N-glycosylase, 8-oxoguanine, alkA protein, and other broad and narrow spectrum DNA glycosylase.

18. (New) The method of claim 15, wherein said surface treatment solution comprises Reacti-Bind.

19. (New) The method of claim 14, wherein the subjecting and tagging steps occur after the binding step.

20. (New) The method of claim 1, wherein the reacting step occurs before the separately mixing step.

oxidative damage. The number of AP sites is quantified by measuring HRP chromogenic substance by an ELISA method.

Alternatively, the sample and control DNA may be tagged or labeled separately with a biotin residue of the ARP reagent, and then bound to the analysis plate for comparison. That is to say, for example, a method of the invention may be practiced by reacting ARP with AP sites of DNA of cells in culture before binding the DNA to the analysis plate since ARP is selectively permeable to cell membranes. Once sample DNA and control DNA are tagged while in culture, they are then extracted, isolated, purified, and bound to the plate for further analysis in accordance with the embodiments described herein. This "in culture" ARP reaction provides even greater sensitivity in detecting abasic sites because background noise is completely removed before other steps of the methods even begin.

The assay method described herein provides an accurate, rapid and cost-effective way to count abasic (AP) sites and DNA (deoxyibrose nucleate acid) base modifications in genomic DNA of cells and tissues. Measurements are performed directly, rather than indirectly, and are performed completely on an analysis plate, such as a commercially available microtiter plate, without the need to remove and/or transport samples to other laboratory facilities. Once DNA samples are purified, an assay may be completed within a few hours using methods and apparatuses of the present invention. Apart from the description contained herein, certain aspects of the invention claimed hereby may be found in recent publications predicated on research of the inventor hereof, including Dojindo Newsletter Vol. 2, entitled Oxidative Stress, DNA Damage and Human Diseases published in the year 2000 by Dojindo Molecular Technologies of Gaithersburg, Maryland, and Technical Manual: DNA Damage Quantification Kit - AP Site Counting, Dojindo Product Code AK02-12, also found at www.dojindo.com, each of which are incorporated herein by reference.

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Deleted: at www.dojindo.com/newsletter/review_vol2.html

6345

PTO/SB/21 (08-00)
Approved for use through 10/31/2002. OMB 0651-0031
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/741,426
Filing Date	12/21/2000
First Named Inventor	Dare, Akintade
Group Art Unit	1634
Examiner Name	Goldberg, Jeanine Anne
Total Number of Pages in This Submission: 23	Attorney Docket Number: MHK.049.001

ENCLOSURES *(check all that apply)*

- [x] Fee Transmittal Form
  - [x] Fee Attached
- [x] Amendment / Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [x] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Response to Missing Parts/ Incomplete Application
  - [ ] Response to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Assignment Papers *(for an Application)*
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) ______
- [ ] After Allowance Communication to Group
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to Group (*Appeal Notice, Brief, Reply Brief*)
- [ ] Proprietary Information
- [ ] Status Letter
- [ ] Other Enclosure(s) *(please identify below)*:

Remarks

Applicant requests an automatic 3-month extension of time under 37 CFR Sec. 1.136(a).

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm *or* Individual name	Lawrence Harbin McIntyre Harbin & King
Signature	[signature]
Date	08/22/2002

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231 on this date: 8/22/2002

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FEE TRANSMITTAL for FY 2002

Patent fees are subject to annual revision.

TOTAL AMOUNT OF PAYMENT ($) 460.00

Complete if Known	
Application Number	09/741,426
Filing Date	12/21/2000
First Named Inventor	Dare, Akintade
Examiner Name	Goldberg, Jeanine Anne
Group Art Unit	1634
Attorney Docket No.	MHK.049.001

METHOD OF PAYMENT

1. ☐ The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:

Deposit Account Number:

Deposit Account Name:

☐ Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17

☑ Applicant claims small entity status. See 37 CFR 1.27

2. ☑ **Payment Enclosed:**

☑ Check ☐ Credit card ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	740	201	370	Utility filing fee	
106	330	206	165	Design filing fee	
107	510	207	255	Plant filing fee	
108	740	208	370	Reissue filing fee	
114	160	214	80	Provisional filing fee	

SUBTOTAL (1) ($)

2. EXTRA CLAIM FEES

			Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X		=	
Independent Claims		- 3** =		X		=	
Multiple Dependent						=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	84	202	42	Independent claims in excess of 3
104	280	204	140	Multiple dependent claim, if not paid
109	84	209	42	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	920	217	460	Extension for reply within third month	460.00
118	1,440	218	720	Extension for reply within fourth month	
128	1,960	228	980	Extension for reply within fifth month	
119	320	219	160	Notice of Appeal	
120	320	220	160	Filing a brief in support of an appeal	
121	280	221	140	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,280	241	640	Petition to revive - unintentional	
142	1,280	242	640	Utility issue fee (or reissue)	
143	460	243	230	Design issue fee	
144	620	244	310	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR 1.17(q)	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	740	246	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	740	249	370	For each additional invention to be examined (37 CFR § 1.129(b))	
179	740	279	370	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) ____________

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

SUBMITTED BY				Complete *(if applicable)*	
Name *(Print/Type)*	Lawrence Harbin	Registration No. *(Attorney/Agent)*	27,644	Telephone	202.408.2779
Signature	[signature]			Date	08/22/2002

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